

## A MULTI-STRAIN PROBIOTIC COMPLEX AS A PART OF A MULTI-APPROACH STRATEGY IN PREVENTING CARDIOVASCULAR RISKS



KEYWORDS: Probiotics, Microbiota, Metabolic Syndrome, Cardiovascular prevention, Weight Management, peer reviewed.

# ABSTRACT

Metabolic syndrome is defined as a cluster of conditions that together concur to increase the cardiovascular risk; its development is the wake-up call for obesity and other cardio metabolic diseases now considered as the major public burden. Microbiota seems to play a key role in the metabolic syndrome, since a dysbiotic status can compromise some cardio-metabolic receptors increasing the incidence of cardiovascular disease risks. Few clinical trials reported positive results, but most of them only focused on cholesterol reduction without mentioning other important parameters as visceral fat or blood pressure. From this perspective, *L. plantarum* PBS067, *L. reuteri* PBS072 and *L. acidophilus* PBS066, have been extensively studied in-vitro and clinically, demonstrating a beneficial effect in terms of waist circumferences, visceral fat and quality of life, providing not only a short-term effect on body shape, but a more comprehensive pro-healthy approach to prevention, delivering long-term outcomes through local microbiota modulation.

### INTRODUCTION

In recent years, it has been defined that gut dysbiosis is not only connected to the development of intestinal symptoms, but also in cardio-metabolic conditions as non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome (MetS) (2–4), which cause a huge impact on both public health and people's quality of life (5). Metabolic syndrome is a multifactorial condition which includes obesity, dyslipidemia, glucose intolerance and hypertension, with a two-fold increased incidence of the risk of coronary heart disease (CHD) and cerebrovascular disease (6).

The growing prevalence of MetS (approximately 31% in the global population) in the last decades underlines the need of new strategies for its prevention and treatment (7, 6). The connection between gut microbiota imbalance, inflammation and its role in the pathogenesis of MetS components is now under investigation (8). Indeed, intestinal microorganisms are involved in several metabolic processes and in the production of bioactive molecules (e.g., short-chain fatty acids, vitamins and metabolites) important for human physiology (9). In this regards, gut microbiota manipulation could represent a promising approach for the prevention and management of metabolic diseases (10–12). For this reason, nutritional strategies aimed at restoring the microbiota have to be addressed from a global perspective, considering

not only the gut but also other extra intestinal targets of action (12).

In the present study the potential effects of a probiotic supplementation on MetS subjects has been assessed, with a randomized, double-blind, placebo-controlled, parallel group, clinical trial.

### METHODS

#### Study design and participants

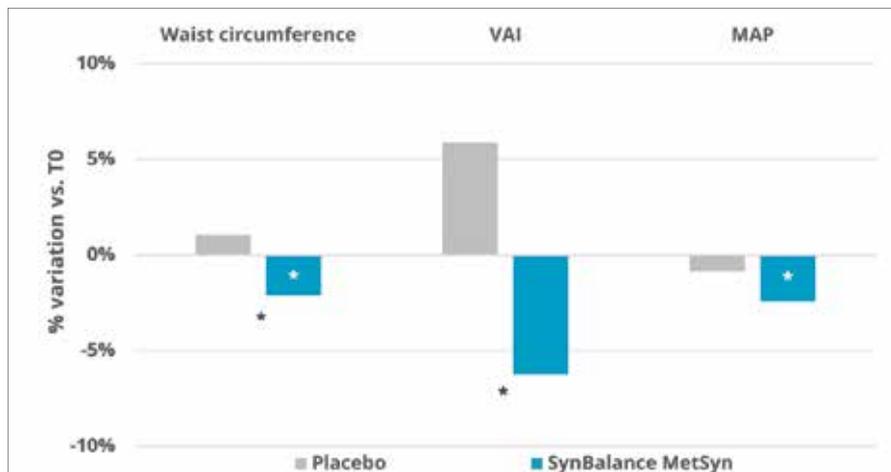
This was a double-blind, randomized, placebo-controlled, parallel-group clinical (RDBPC) trial carried out in a group of adults with metabolic syndrome according to International Diabetes Federation (IDF) criteria (13) with no other disabling conditions. The study included a 14-day run-in period of diet standardization and a 60-day treatment period. During the enrolment, a specialist physician developed flexible diet plans and encouraged volunteers to increase their physical activity. Patients were evaluated clinically and blood samples were collected at the beginning and at the end of the trial. In addition, subjects were asked to complete the EuroQol- 5 Dimension (EQ-5D) questionnaire. The study fully complied with the ethical guidelines of the Declaration of Helsinki and with The International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Harmonized Tripartite Guideline for Good Clinical Practice (GCP). The study protocol was approved by the Ethical Committee of the University of Bologna.

All volunteers signed a written informed consent to participate and were free to withdraw from the study at any time.

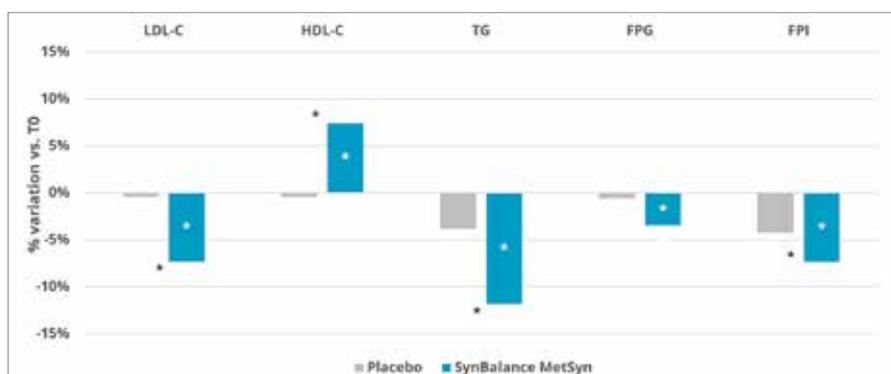
#### Treatment

A randomized, double-blind, controlled clinical trial on 60 (30 active and 30 placebo) subjects diagnosed with metabolic syndrome was carried out. Baseline characteristics were similar in the two groups in terms of age ( $71 \pm 3$  placebo and  $72 \pm 3$  active group), BMI ( $27.3 \pm 2.5$  placebo and  $27.4 \pm 2.6$  active group) and gender (13 male and 17 female in the placebo group while 14 male and 16 female in the active group).

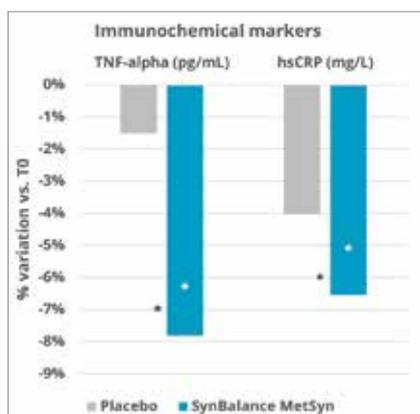
Following 14 days of standardization, each patient was randomly allocated to either placebo or active treatment, they were asked to take one liquid vial every day for 60 days. Any differences between the two products were observed. *Lactobacillus plantarum* PBS067, *Lactobacillus acidophilus* PBS066 and *Lactobacillus reuteri* PBS072 with a final concentration of  $6 \times 10^9$  CFU,  $2 \times 10^9$  CFU for each strain, were enclosed in the dosing cap, while prebiotic fibers, 30 mg of inulin and 30 mg of fructooligosaccharides (FOS) were dissolved in the liquid phase. All patients were recommended to take the treatment every day early in the morning. Participants' compliance was evaluated by counting the number of vials returned at the last visit of the trial.



**Figure 1.** Anthropometric evaluation. Improvement of anthropometric parameters as Waist circumference, Visceral Adipose Index (VAI) and Mean Arterial Pressure (MAP). Results are reported as a variation with respect to the baseline (T0) and are reported in percentage. White stars: result has a p value <0.05 with respect to the T0; Black stars: the result has a p value <0.05 with respect to the placebo.



**Figure 2.** Ematochemical profile. Results are reported as a variation with respect of the beginning of treatment in percentage. Positive results were observed for LDL, HDL cholesterol and Tryglicerides as well as for Fasting Plasma Glucose (FPG) and Fasting Plasma Insulin (FPI). White stars: result has a p value <0.05 with respect to the T0; Black stars: the result has a p value <0.05 with respect to the placebo.



**Figure 3.** Inflammatory markers. Modulation of the inflammatory markers with respect to placebo. Results are reported as a variation towards the baseline and expressed as a percentage. Positive results observed for Tumor Necrosis Factor – Alpha (TNF- $\alpha$ ) and High-Sensitive C Reactive Protein (hs-CRP). White stars: result has a p value <0.05 with respect to the T0; Black stars: the result has a p value <0.05 with respect to the placebo.

### Assessments

Patients' personal history was evaluated taking particular attention to CVD and other diseases, dietary and smoking habits assessment (14), physical activity and pharmacological treatment. Primary outcome was linked to the amelioration of the criteria connected with the development of Metabolic syndrome as waist circumference, visceral fat, lipid profile and blood pressure.

Waist circumference (WC) was measured at the end of a normal expiration, in a horizontal plane at the midpoint between the inferior margin of the last rib and the superior iliac crest. Body mass index (BMI) was calculated as body weight in kilograms, divided by height squared in meters ( $\text{kg}/\text{m}^2$ ) (15).

The EQ-5D questionnaire is a descriptive system with one question for each of the five dimensions that include mobility, self-care, usual activities, pain/ discomfort, and anxiety/depression, able to evaluate subjects' quality of life. The questionnaire includes a Visual Analog Scale (VAS), by which respondents can report their status with a grade ranging from 0 (the worst possible) to 100 (the best possible) (16).

Blood samples for biochemical analysis were carried out in order to detect the modulation of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), fasting plasma insulin (FPI), high-sensitivity C- reactive protein (hsCRP), tumor necrosis factor alpha (TNF-alpha), and visceral adiposity index (VAI). TNF-alpha was measured by enzyme-linked immunosorbent assay (ELISA). LDL-C was obtained by the Friedewald formula. Non- HDL-C resulted from the difference between TC and HDL-C (17). Systolic (SBP) and diastolic blood pressure (DBP) measurements were detected in each subject supine and at rest, by the use of a standard mercury, with a cuff of the appropriate size applied to the right upper arm.

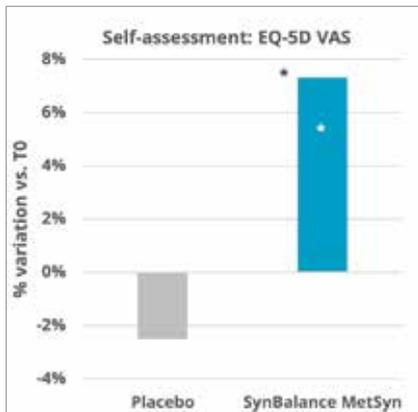
During the clinical trial, volunteers were carefully monitored for any adverse event.

### STATISTICAL ANALYSIS

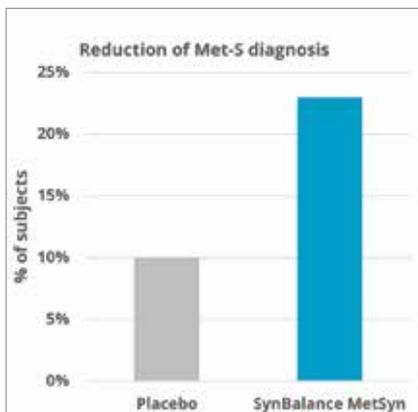
Baseline between group characteristics were compared using the independent t test for normally distributed variables and the Kruskal-Wallis H test for non-parametric variables. Between group differences were assessed by the ANOVA followed by the Tukey's post hoc test. All data were expressed as means and related standard deviations. All tests were two sided. A p level of < 0.05 was considered significant for all tests.

### RESULTS

All participants completed the trial without any adverse event and a similar compliance to the trial (99.7% overall;  $p > 0.05$  between groups).



**Figure 4.** Quality of life questionnaire. Variation with respect to the beginning of the treatment (T0) of the quality of life measured by the EuroQol-5 Dimension questionnaire (EQ-5D), results are reported as a percentage. White stars: result has a p value <0.05 with respect to the T0; Black stars: the result has a p value <0.05 with respect to the placebo.



**Figure 5.** Reduction of MetS diagnosis. Percentage of subjects who do not comply anymore with the criteria of Metabolic Syndrome.

Results after two months showed a significant ( $p < 0.05$ ) global reduction of waist circumference, visceral adipose index (Figure 1), triglycerides, LDL cholesterol and fasting plasma insulin as well as fasting plasma glucose compared to baseline and placebo (Figure 2). HDL cholesterol showed a significant improvement versus baseline and placebo ( $p < 0.05$ ). In addition, there was a significant decrease of hsCRP protein and TNF- $\alpha$ , the principal cytokine involved in inflammation ( $p < 0.05$ ) (Figure 3).

At the end of the study, the number of patients diagnosed with metabolic syndrome was reduced (-23%) in the active group with respect to placebo

(-10%) as well as a significant improvement of patient's quality of life (Figure 4-5).

## DISCUSSION

Metabolic syndrome (MetS) is defined as a cluster of conditions that together concur to increase the cardiovascular risk: it is linked to obesity, insulin resistance, dyslipidemia, hypertension and elevated C reactive proteins (CRP) levels (6). The development of metabolic syndrome is the wake-up call for obesity and other cardio metabolic diseases according to IDF guidelines. MetS is around three times more common than diabetes, with an estimated global prevalence of about the 31% of the world population (5).

The present RDBPC clinical study aims to assess the ability of a probiotic formulation containing *Lactobacillus plantarum* PBS067, *Lactobacillus acidophilus* PBS066 and *Lactobacillus reuteri* PBS072 to exert a positive effect on metabolic syndrome by improving body waist circumference, lipid metabolic profile, chronic inflammation and also overall quality of life.

Following two months of product intake, results showed a significant ( $p < 0.05$ ) global reduction of anthropometric parameters as waist circumference of the waist, visceral fat and blood pressure. At the same time the blood lipid profile revealed a positive modulation of lipid biomarkers as well as insulin and glycaemia. The inflammatory profile was also improved, showing a significant effect of probiotic strains on immune markers involved in the inflammatory cascade. The most important aspect is related to the higher percentage of people that no more comply with MetS criteria in the active group; this result underlines that in a specific condition, sometimes the only improvement of the lifestyles is not enough to achieve significant results, but a multiple approach is recommended.

## CONCLUSION

All together, these results may broaden the area of non-medication strategies in support of MetS, which

currently consist of healthy nutrition (with large quantities of foods high in beneficial antioxidants and polyunsaturated fatty acids) and regular physical activity (8, 18-19).

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## NEWS FROM SCIENCE

### STUDY FINDS DIGITAL TREATMENT FOR OSTEOARTHRITIS IS SUPERIOR TO TRADITIONAL ROUTINE CARE

The steadily increasing prevalence and high costs of treating chronic joint pain worldwide poses a challenge for healthcare systems and healthcare payers. New research shows the effectiveness of a digital healthcare treatment with the potential to save insurance companies and their patients the costs and risks of joint surgeries - a finding that is especially promising as more patients turn to telemedicine as a safe treatment option amid the COVID-19 pandemic.

A new randomized controlled trial (RCT) conducted by the University of Nottingham using Joint Academy's clinical evidence-based digital treatment for chronic joint pain is the first to find clinically important improvements of treating knee osteoarthritis digitally compared to traditional treatment. Patients receiving digital treatment reduced their pain by 41 percent, while patients receiving traditional care only experienced a 6 percent decrease.

"We already knew that digital first-line treatment substantially improves symptoms of osteoarthritis at a significantly lower cost than face-to-face care. This study firmly establishes how effective digital treatment actually is in relation to traditional self-management

care," says Leif Dahlberg, Chief Medical Officer at Joint Academy and Senior Professor in Orthopedics.

A total of 105 people, who were 45 years or older with a diagnosis of knee osteoarthritis, participated in the study. They were allocated at random to two groups. One was treated digitally and the other self-managed their symptoms according to guidelines. Patients in the digital treatment were connected with licensed physical therapists via a smartphone application where they received education and daily exercises. In the other group, patients continued their traditional self-management program and visited their general practitioner when needed.

Osteoarthritis is one of the world's fastest growing and most costly chronic diseases. According to the Centers for Disease Control, more than 32 million U.S. adults are affected. It is also among the most expensive conditions to treat when joint replacement surgery is required. For the millions who suffer with the daily pain and stiffness of osteoarthritis, treatments to slow the progression of the disease are limited. The recommended first-line treatment, consisting of information, exercise and weight control when needed, is underutilized.

**Source:** [www.nottingham.ac.uk](http://www.nottingham.ac.uk), [www.jointacademy.com](http://www.jointacademy.com)